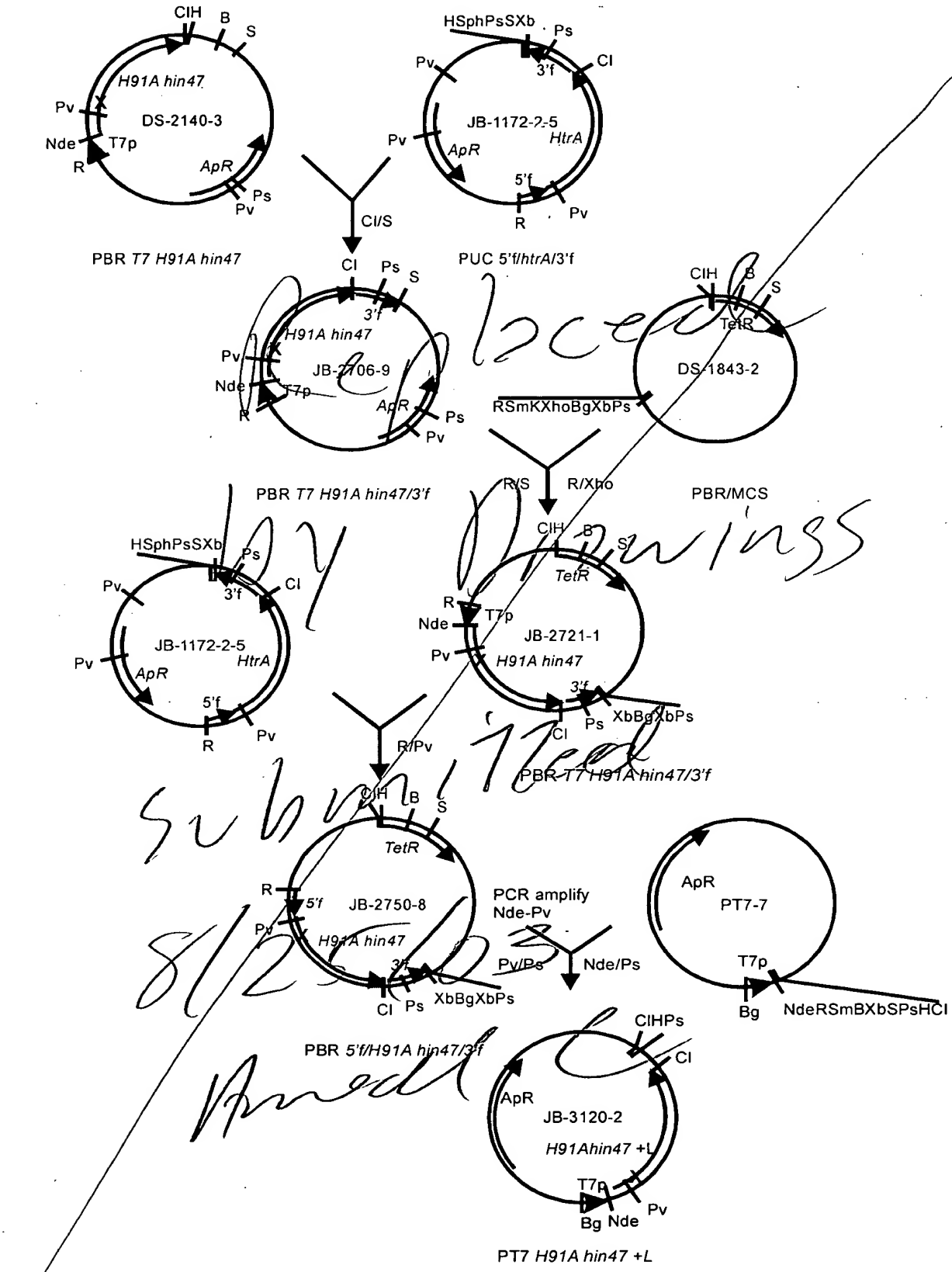
Figure 1A. Constructi[redacted]f JB-3120-2, a plasmid containing T[redacted]A *hin47* (+ leader)



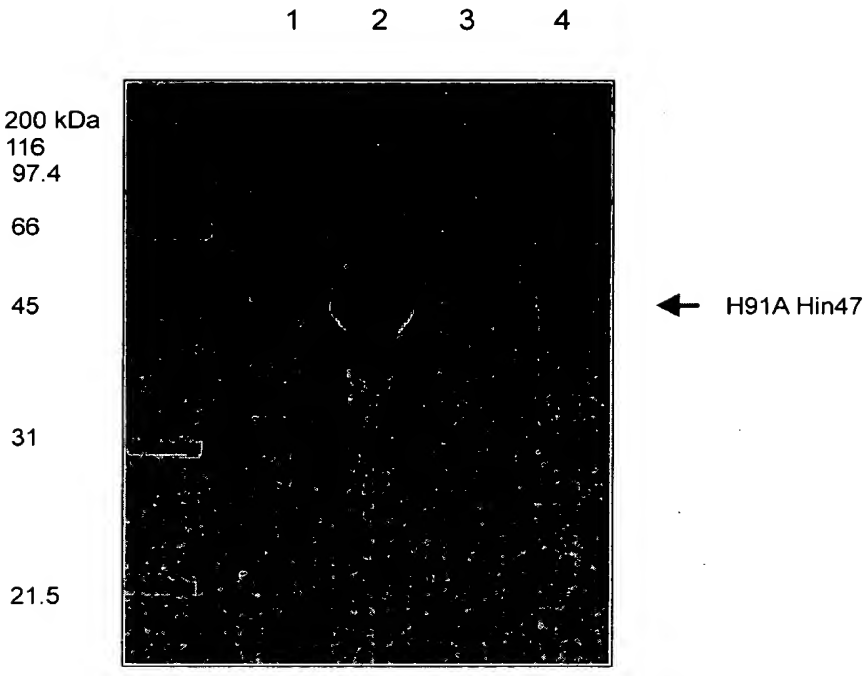
Co-expn fig 1
pJB-3120

Figure 1B. Oligonucleotide primers to PCR amplify the Nde I-Pvu I fragment encoding the leader sequence of HtrA.

```
            Nde I
                M  K  K  T  R  F  V  L  N  S                    SEQ ID No: 2
5'   GGCCGCATATGAAAAAAACACGTTTTGTACTAAATAGT  3'   6931.SL      SEQ ID No: 1


                        Pvu I
     F  K  F  F  F  G  D  R  F  A  E  Q                         SEQ ID No: 5
     TTTAAATTCTTCTTTGGCGATCGTTTTGCCGAACAA                       SEQ ID No: 4
3'   AAATTTAAGAAGAAACCGCTAGCAAAACGGCTTGTT  5'   6932.SL      SEQ ID No: 3
```

Figure 2. Production of H91A Hin47 +/- leader



1. H91A Hin47 (- leader) t_0
2. H91A Hin47 (- leader) t_4
3. H91A Hin47 (+ leader) t_0
4. H91A Hin47 (+ leader) t_4

Co-expn fig 2
HinLexp

Figure 3. Purification of H91A Hin47 (+ leader) from *E. coli*

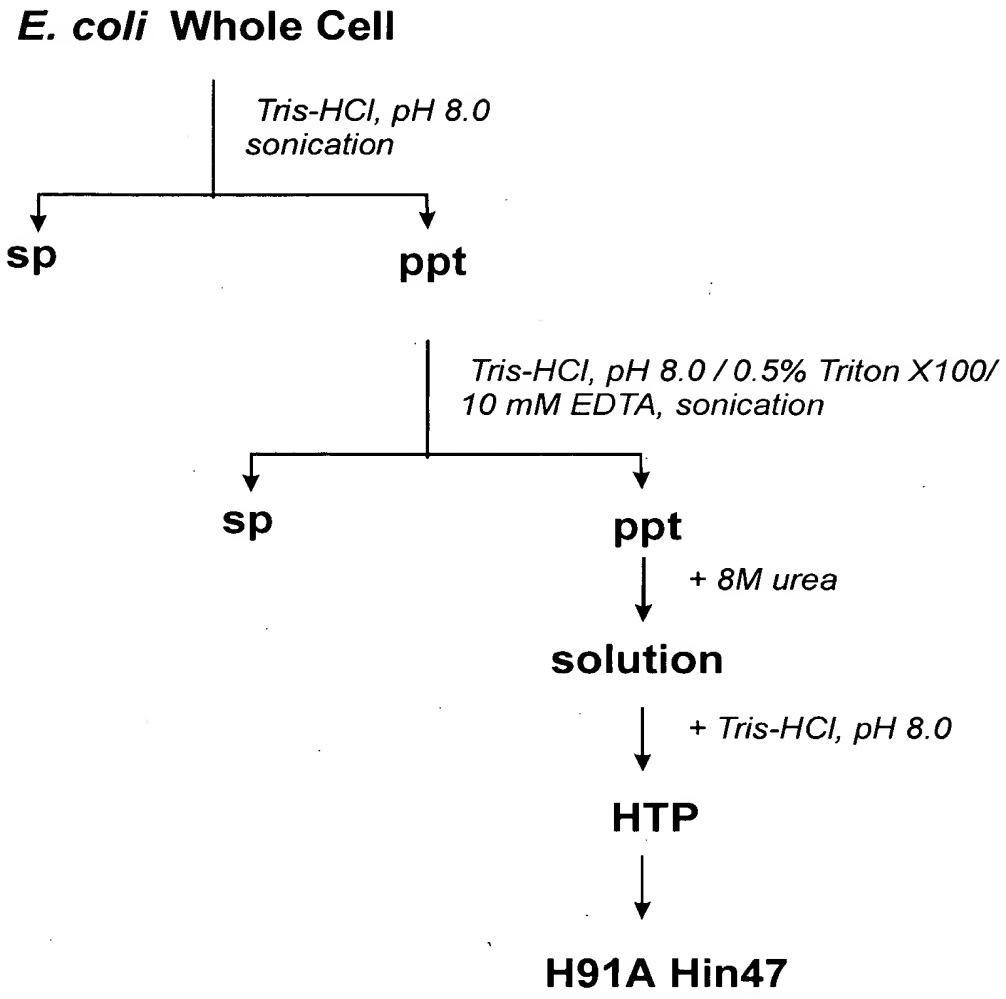
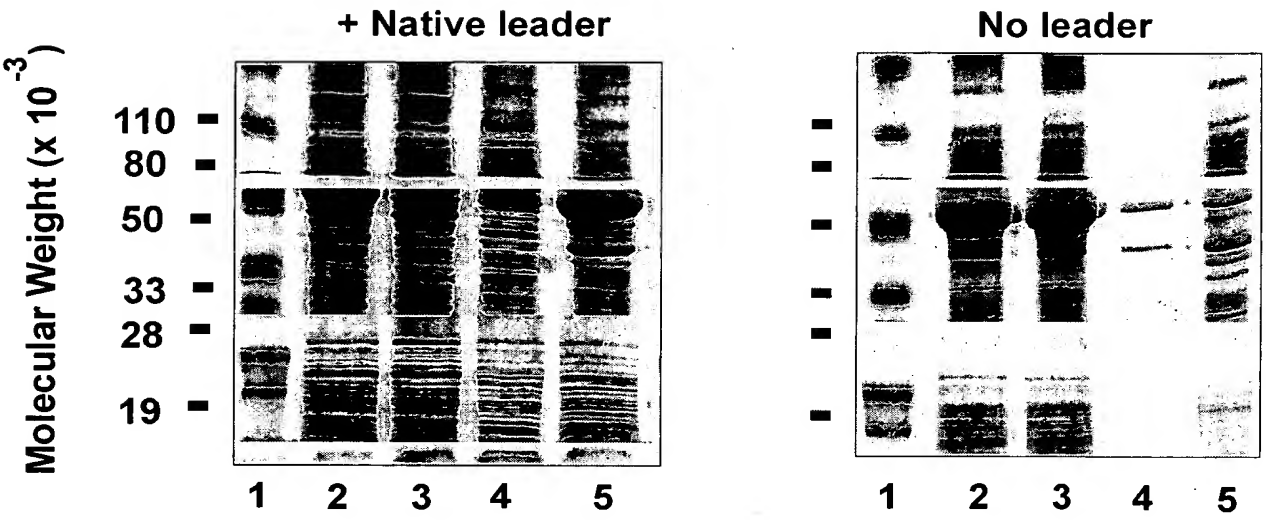
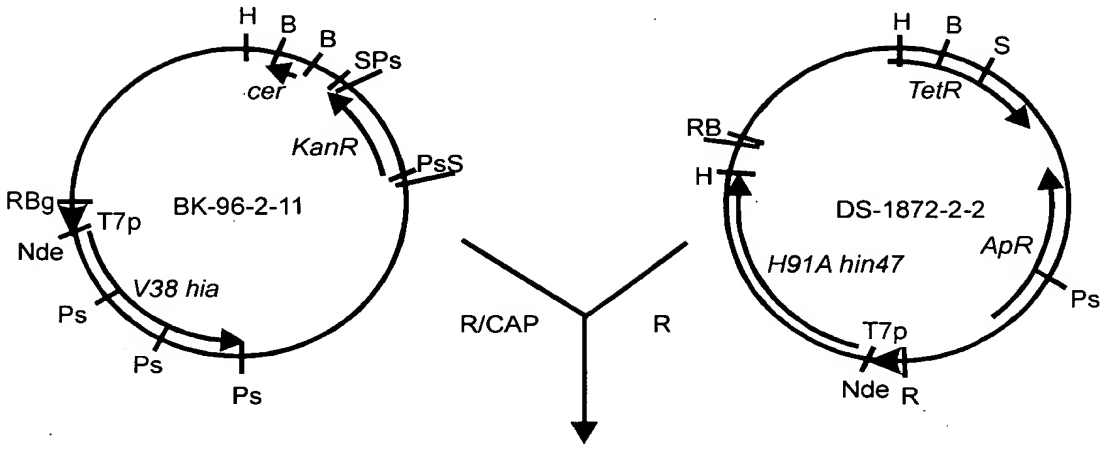


Figure 4. Extraction of H91A Hin47 +/- leader, produced in *E. coli*



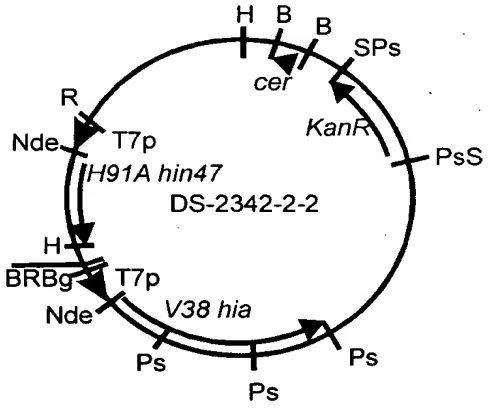
1. Pre-stained markers
2. *E. coli* whole cells
3. Soluble proteins in 50 mM Tris-HCl extraction
4. Soluble proteins in Tris/EDTA/Triton-100 extraction
5. Remaining pellets

Figure 5. Construction of DS-2342-2-2, a plasmid containing *T7 H91A hin47* and *T7 V38 hia*



pBR T7 V38 hia /cer/kanR

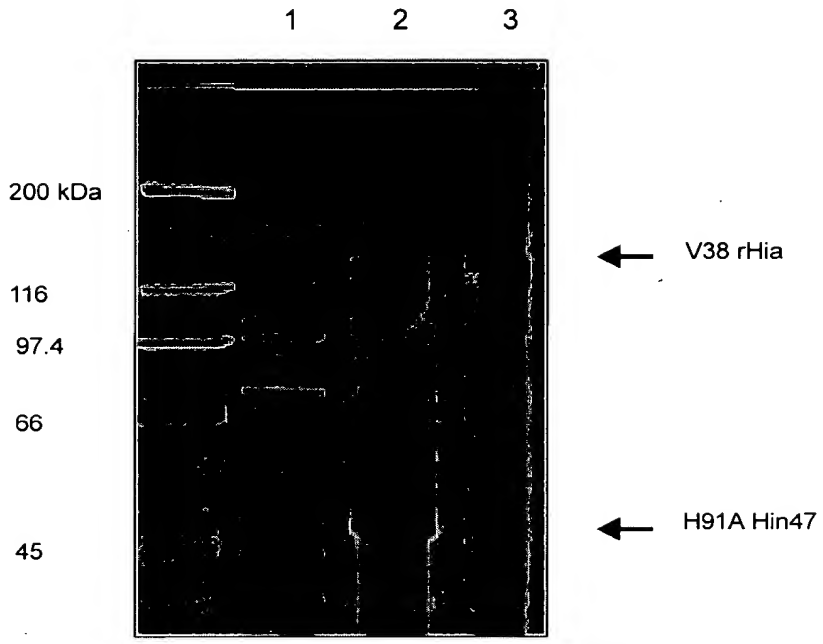
pBR T7 H91A hin47



pBR T7 V38 hia /T7 H91A hin47/cer/KanR

Co-expn fig 5
pDS-2342

Figure 6. Production of V38 rHia and H91A Hin47 when co-expressed



1. V38 rHia and H91A Hin47 t_0
2. V38 rHia and H91A Hin47 t_4
3. V38 rHia alone t_4

Co-expn fig 6
V38hin

Figure 7. Purification of H91A Hin47 and V38 rHia from *E. coli*

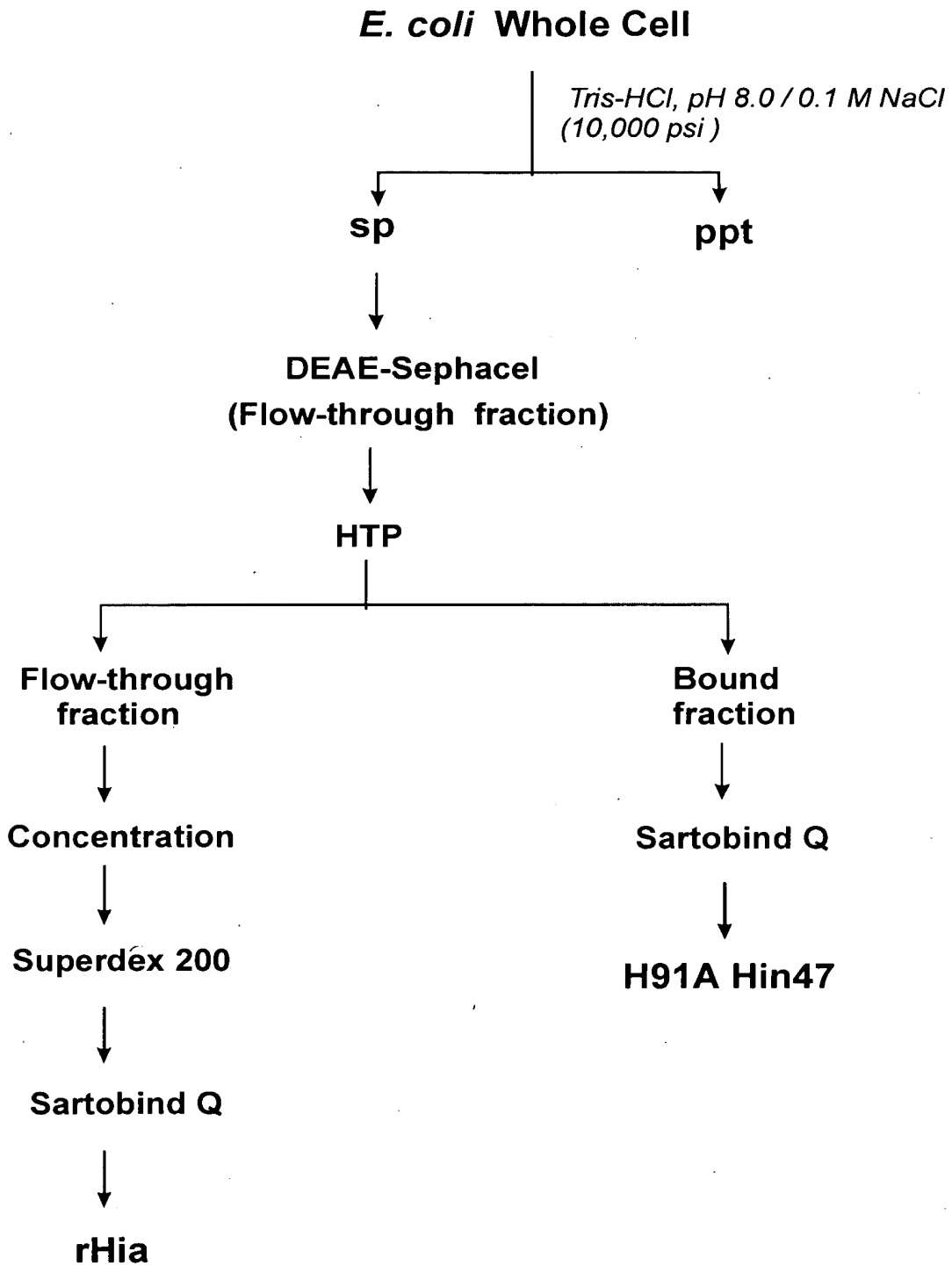
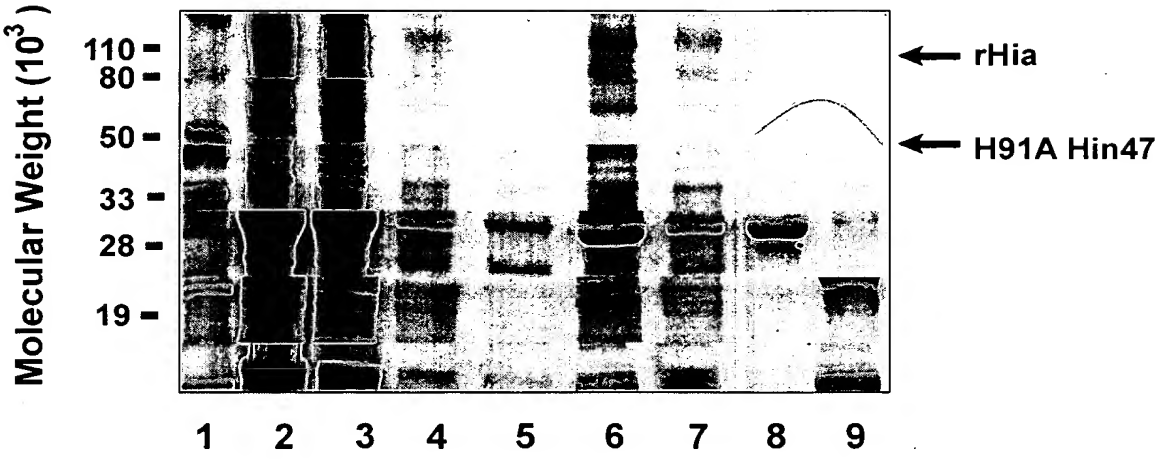
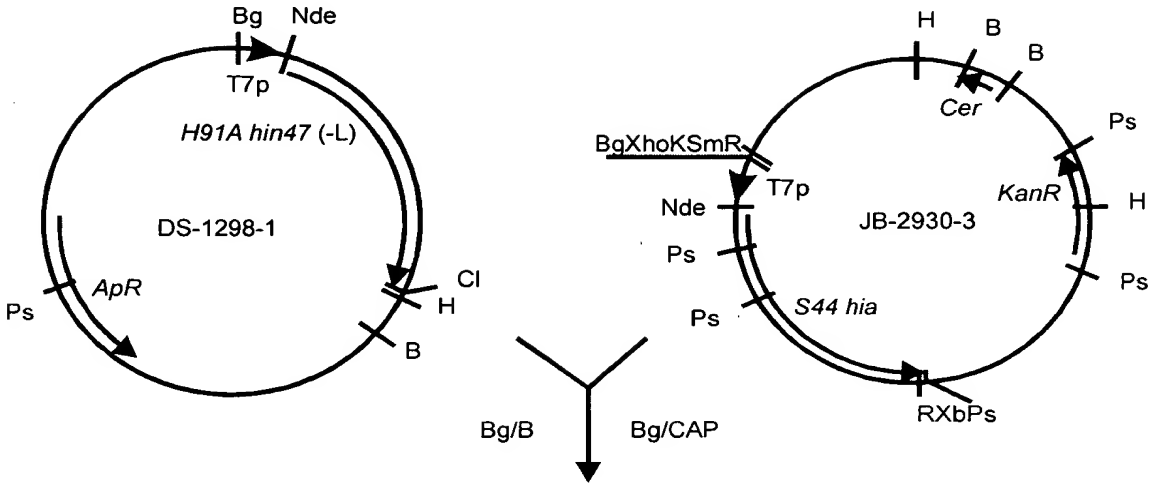


Figure 8. Purification of H91A Hin47 and V38 rHia from *E. Coli*



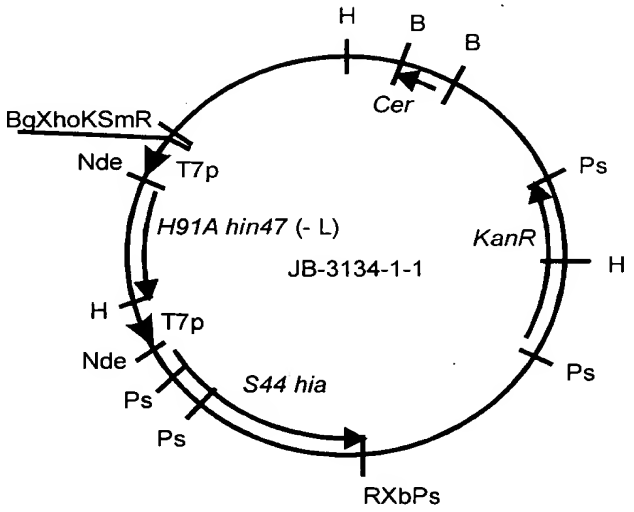
1. **Prestained molecular weight markers**
2. ***E. coli* whole cell lysate**
3. **Tris/NaCl extract**
4. **Tris/ Triton X-100 / EDTA extract**
5. **Tris/ OG extract**
6. **DEAE flow-through**
7. **HTP flow-through**
8. **Purified H91A Hin47**
9. **Purified rHia**

Figure 9. Construction of JB-3134-1-1, a plasmid containing T7 *H91A hin47* (- leader) and T7 *S44 hia*



PEV T7 *H91A hin47* (- L)

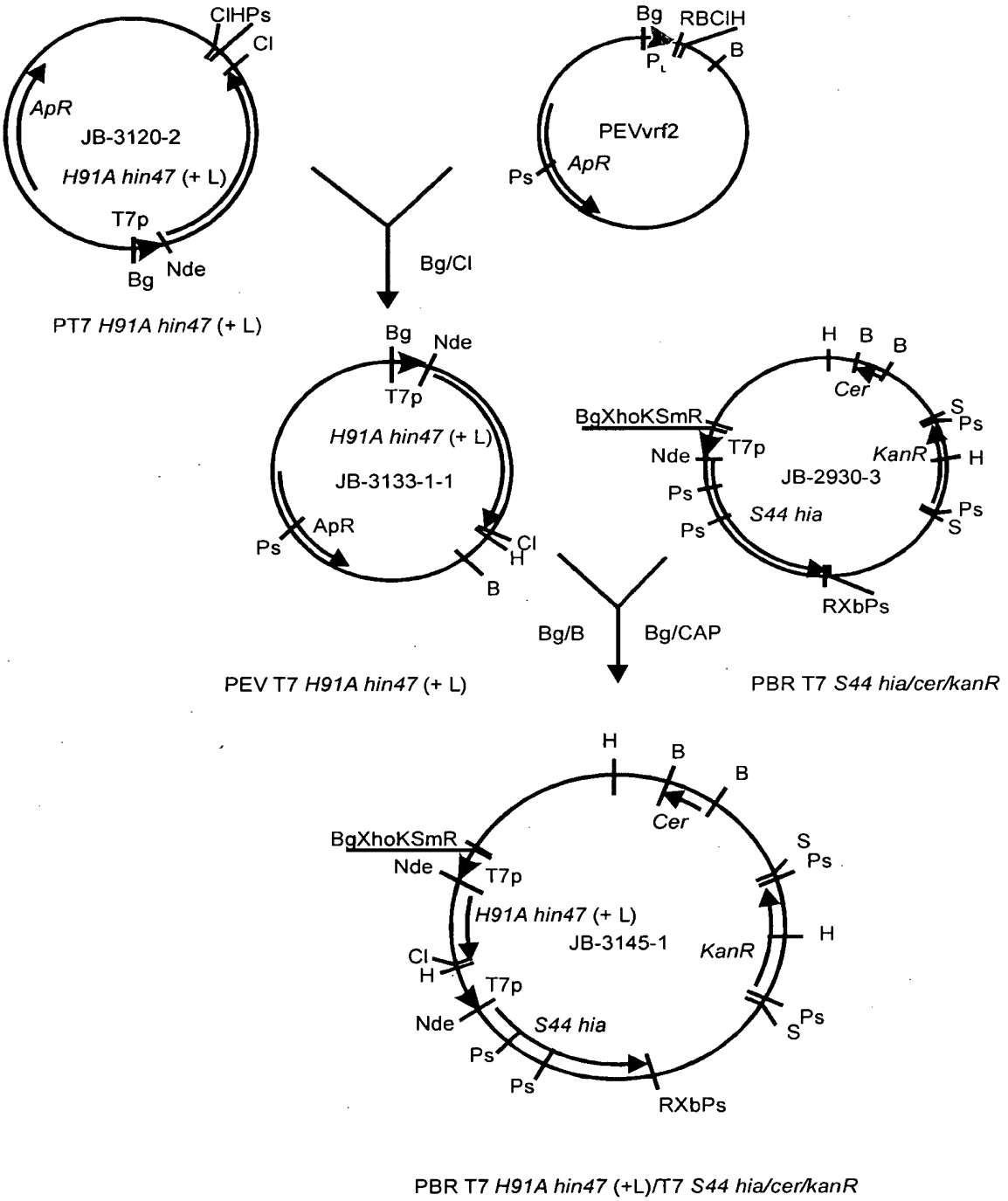
PBR T7 *S44 hia/cer/kanR*



PBR T7 *H91A hin47* (- L)/T7 *S44 hia/cer/kanR*

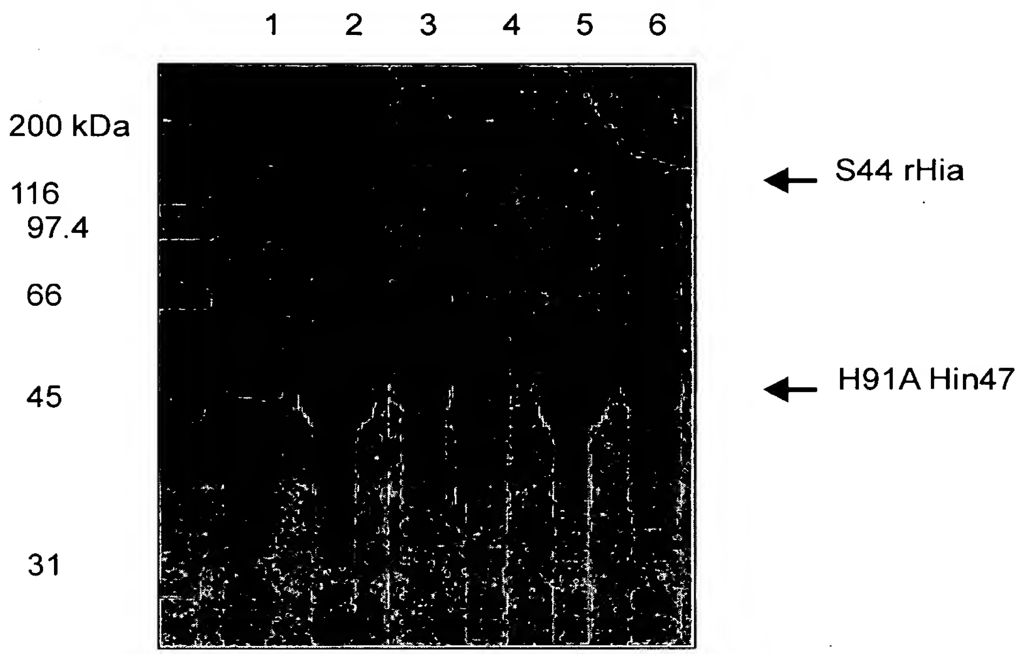
Co-expn fig 9
pJB-3134

Figure 10. Construction of JB-3145-1, a plasmid containing T7 *H91A hin47* (+ leader) and T7 *S44 hi*



Co-expn fig 10
pJB-3145

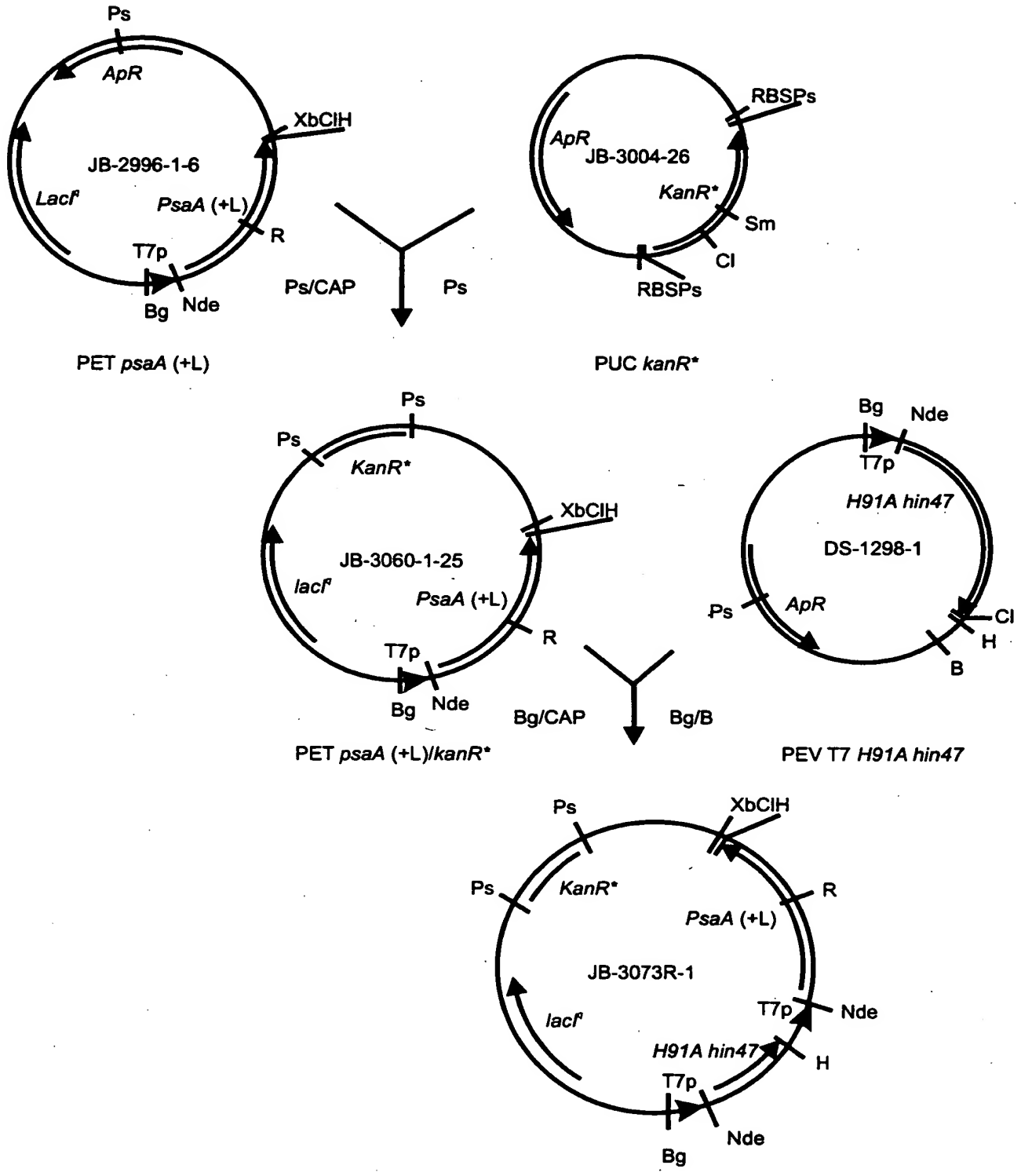
Figure 11. Production of S44 rHia and H91A Hin47 (+/-L), when co-expressed.



1. H91A Hin47 (-L) t_0
2. H91A Hin47 (-L) t_4
3. H91A Hin47 (+L) t_4
4. S44 rHia t_4
5. H91A Hin47 (-L) + S44 rHia t_4
6. H91A Hin47 (+L) + S44 rHia t_4

Coexpn fig 11
S44HinL

Figure 12A. Construction of JB-[redacted]3R-1, a plasmid containing T7 *psaA* [redacted]leader) and T7 *H91A hin47*



Co-expn fig 12A
pJB-3073

Figure 12B. Oligonucleotide primers to PCR amplify psaA (+ native leader)

Sense:

```
                Nde I
                   M  K  K  L  G  T  L  L  V  L
5'   CGGGATCCCATATGAAAAAATTAGGTACATTACTCGTTCTC   3'    6850.SL
```

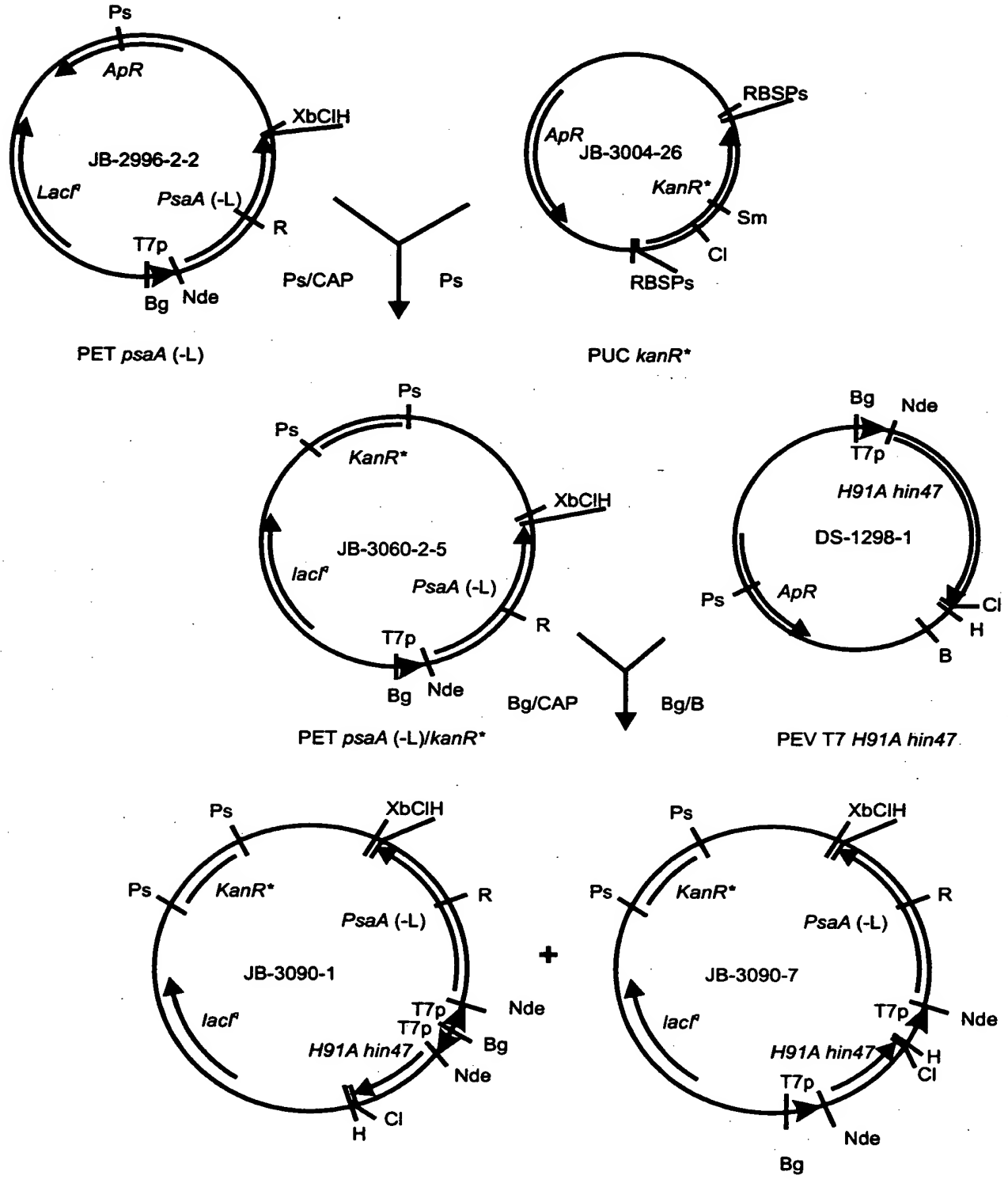
SEQ ID NO: 27
SEQ ID NO: 26

Antisense:

```
     CATGTGAGCTGGCGTTTTTTAAGCTTGGCC
3'   GTACACTCGACCGCAAAAAATTCGAACCGG          5'    6852.SL
                        Hind III
```

SEQ ID NO: 29
SEQ ID NO: 28

Figure 13A. Construction of JB-3090-1 and JB-3090-7 plasmids containing T7 *psaA* (- leader) and T7 *H91A hin47*



Co-expn fig 13A
pJB-3090

Fig 13B. Oligonucleotide primers to PCR amplify psaA (- leader)

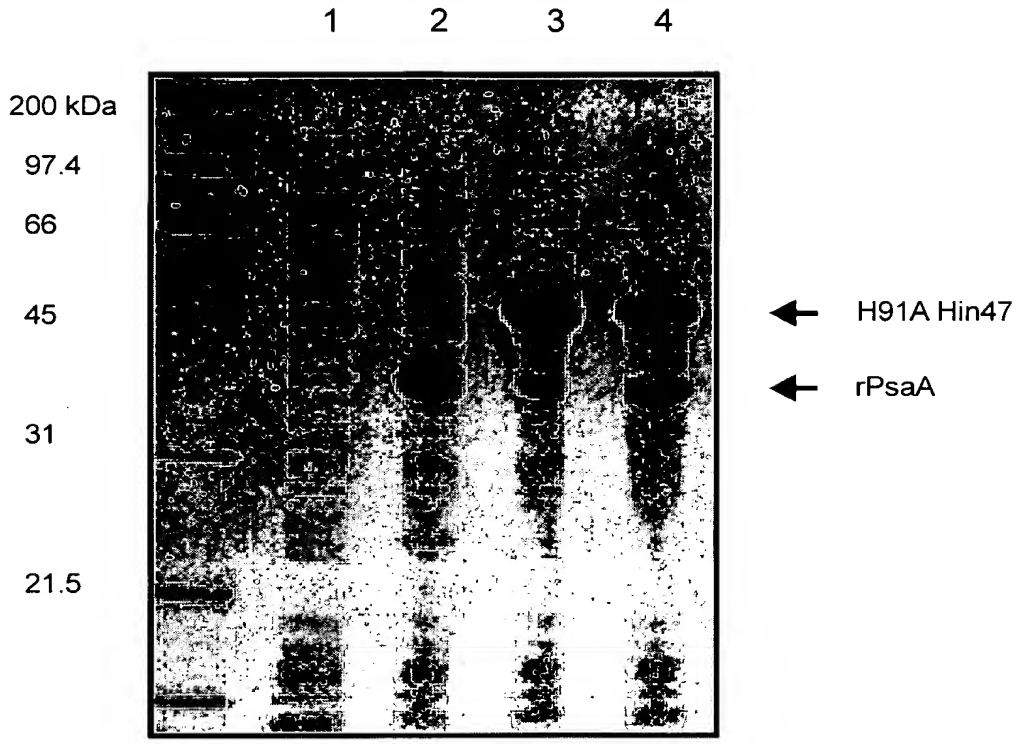
Sense:

```
                    M  C  A  S  G  K  K  D  T                                    SEQ ID NO: 31
5'   CGGGATCCCATATGTGTGCTAGCGGAAAAAAAGATACA      3'    6851.SL                   SEQ ID NO: 30
```

Antisense:

```
     CATGTGAGCTGGCGTTTTTTAAGCTTGGCC                                               SEQ ID NO: 29
3'   GTACACTCGACCGCAAAAAATTCGAACCGG      5'    6852.SL                           SEQ ID NO: 28
                         Hind III
```

Figure 14. Production of rPsaA +/- leader and H91A Hin47, when co-expressed



1. H91A Hin47 and rPsaA (+ leader), < > orientation t_0
2. H91A Hin47 and rPsaA (+ leader), < > orientation t_4
3. H91A Hin47 and rPsaA (+ leader), > > orientation t_4
4. H91A Hin47 and rPsaA (- leader), > > orientation t_4

Co-expn fig 14
psaahin

Figure 15. Purification of H91A Hin47 and rPsaA from *E. coli*

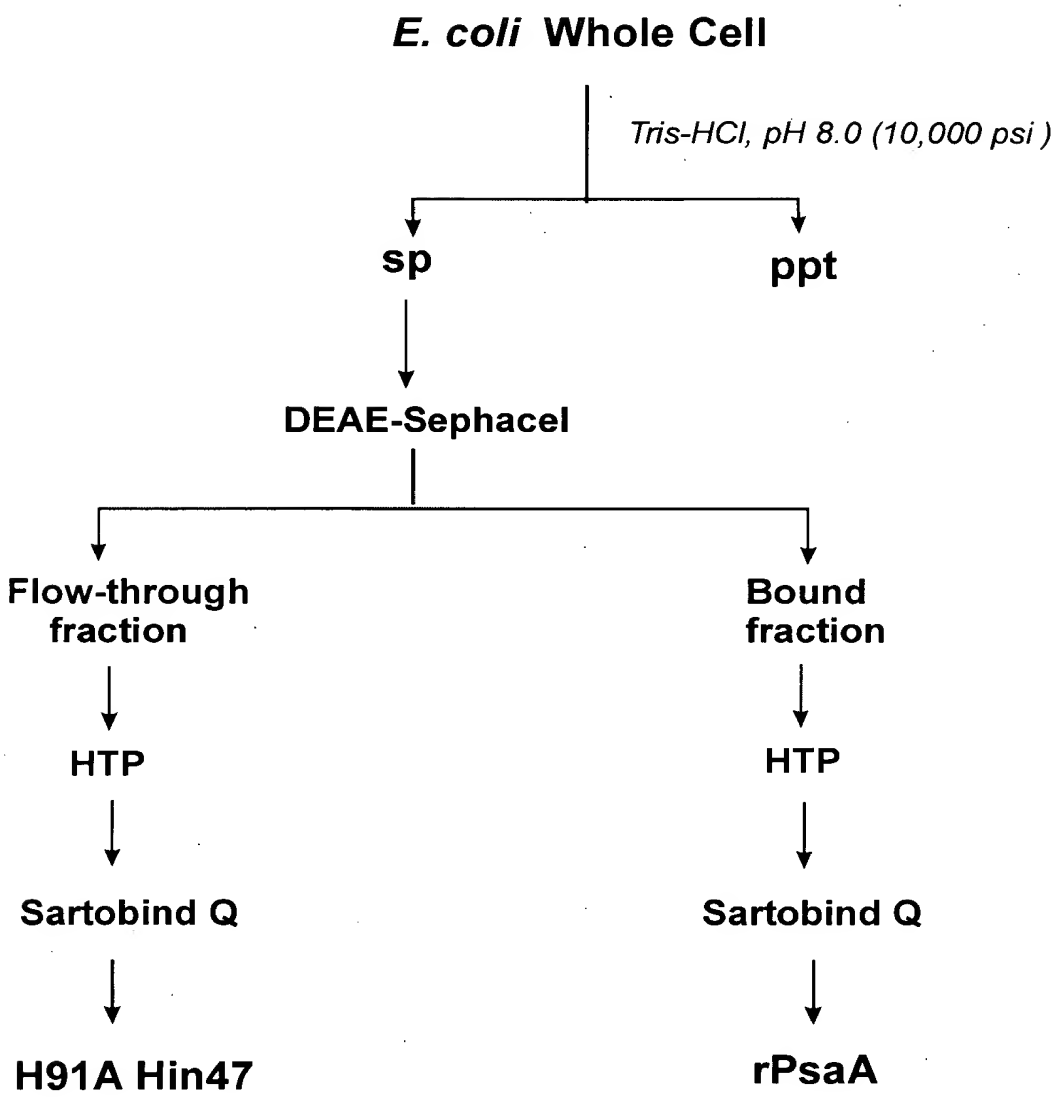
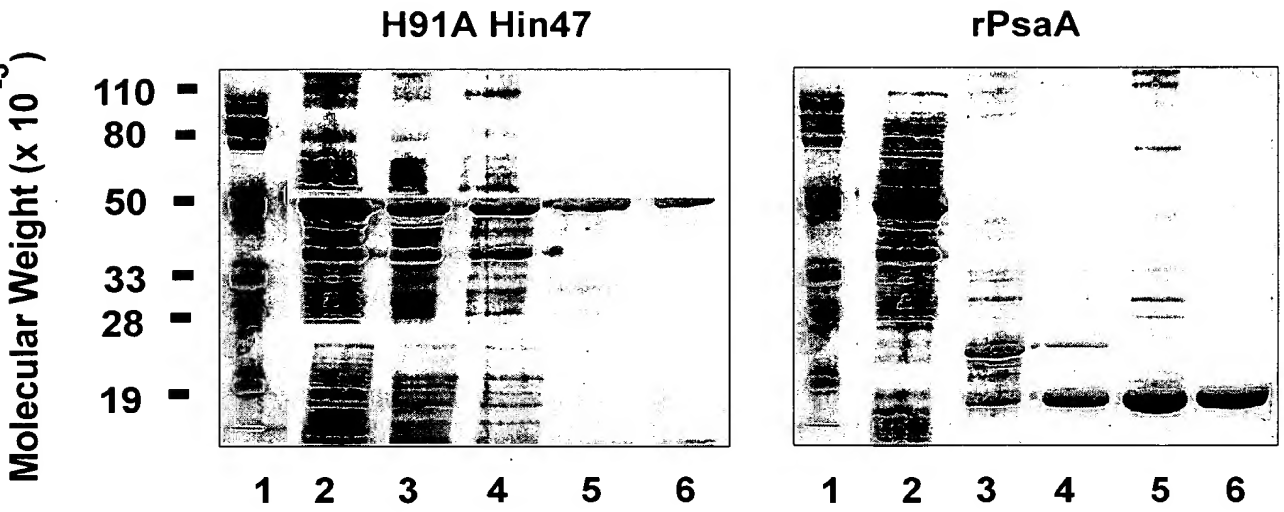
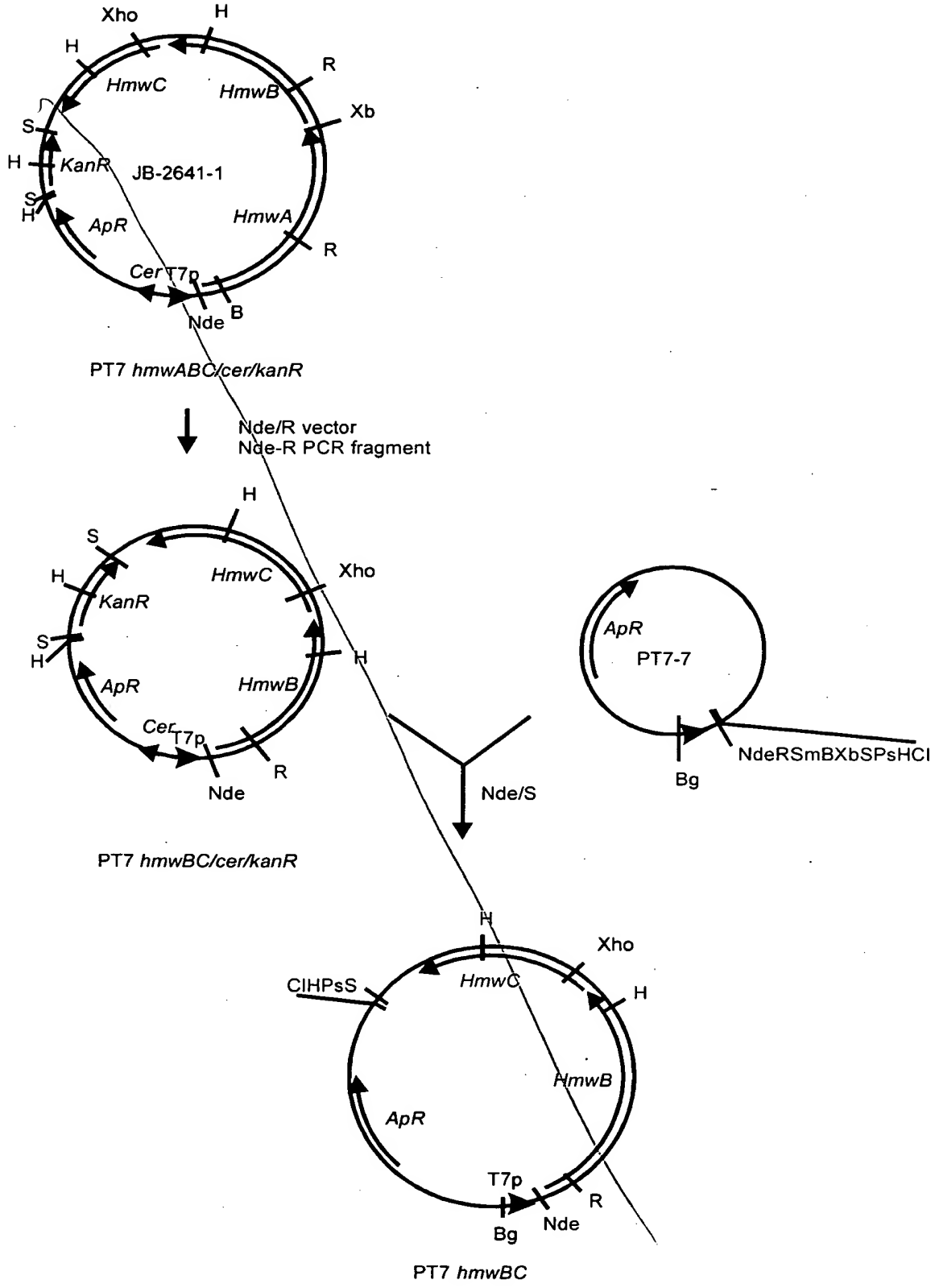


Figure 16. Purification of H91A Hin47 and rPsaA from *E. coli*



1. **Pre-stained molecular weight markers**
2. ***E. coli* whole cell lysate**
3. **Soluble proteins in 50 mM Tris-HCl, pH 8.0 extraction**
4. **DEAE-Sephacel column**
5. **HTP column**
6. **Sartobind Q membrane**

Figure 17A. Construction of a plasmid containing *T7 hmwBC*.



Co-expn fig 17A
phmwBC

Figure 17B. Oligonucleotide primers for PCR amplification of Nde I-EcoR I *5' hmwB* fragment.

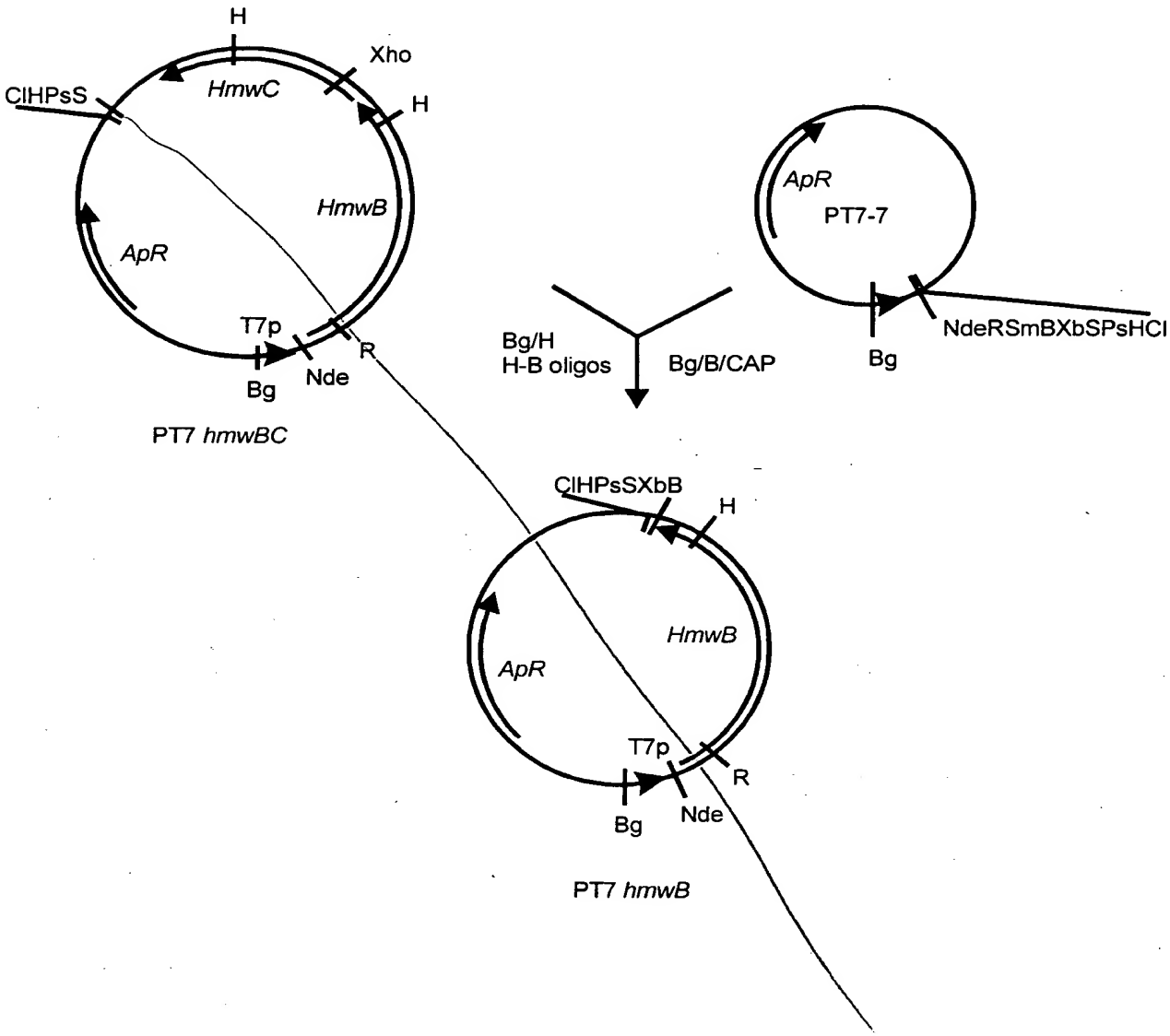
coding strand:

```
            Nde I
              M  K  N  I  K  S  R  L  K  L                    SEQ ID No: 7
5'   GGCCGCATATGAAAAATATAAAAAGCAGATTAAAACTC   3'   7072.SL   SEQ ID No: 6
```

non-coding strand:

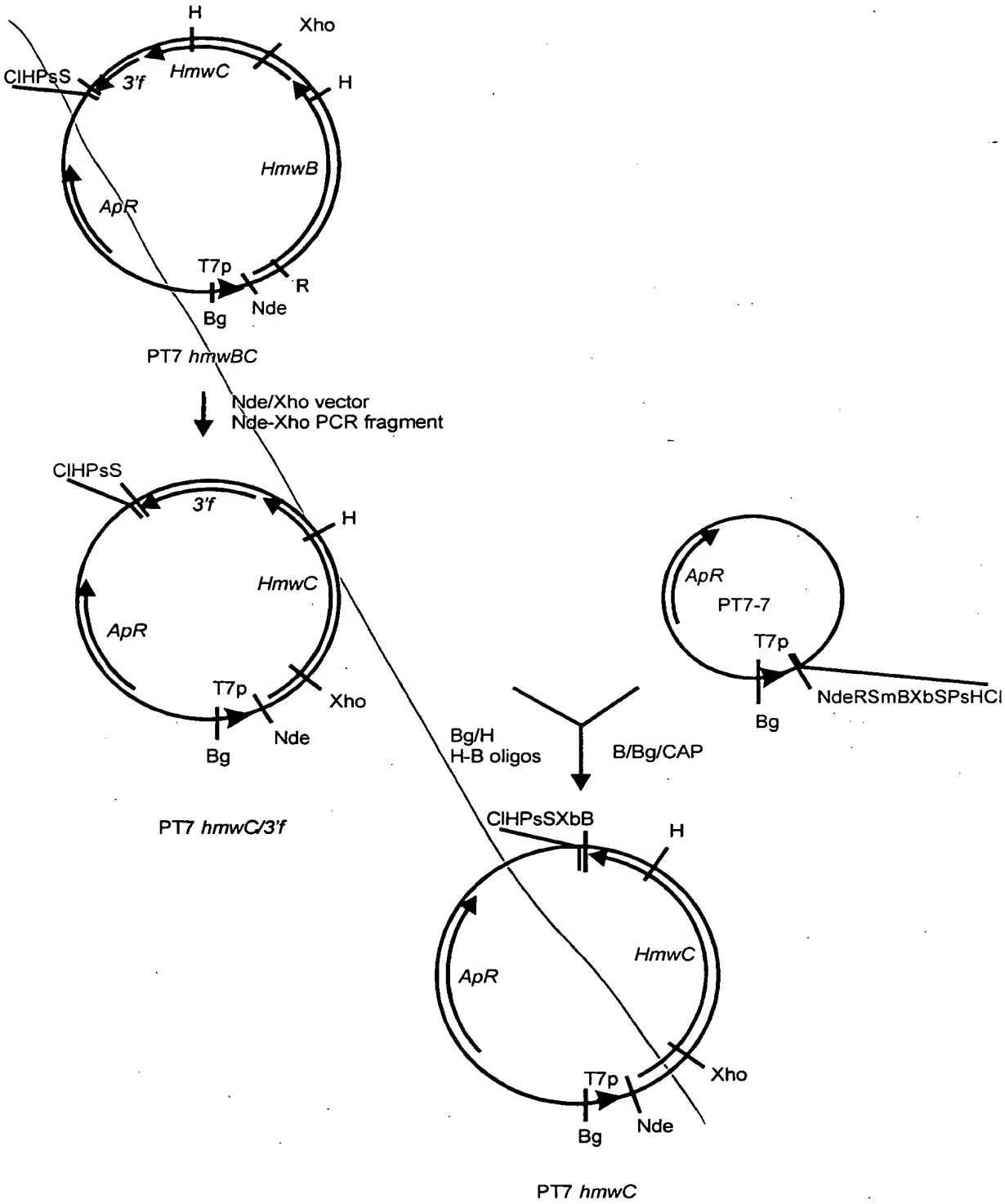
```
                              EcoR I
     G  R  Q  W  F  D  L  R  E  F  N  M  A                    SEQ ID No:10
     GGTCGTCAGTGGTTCGATTTGCGTGAATTCAATATGGCA                  SEQ ID No: 9
3'   CCAGCAGTCACCAAGCTAAACGCACTTAAGTTATACCGT   5'   5950.SL   SEQ ID No: 8
```

Figure 18A. Construction of a plasmid containing *T7 hmwB*.



Co-expn fig 18A
phmwB

Figure 19A. Construction of a plasmid containing *T7 hmwC*.



Co-expn fig 19A
phmwC

Figure 19B. Oligonucleotide primers for PCR amplification of Nde I-Xho I *5' hmwC* fragment.

coding strand:

```
              Nde I
                 M  T  K  E  N  L  Q  S  V  P                          SEQ ID No:17
5'    GGCCGCATATGACAAAAGAAAATTTACAAAGTGTTCCA    3'    7077.SL    SEQ ID No:16
```

non-coding strand:

```
                           Xho I
       S  T  S  M  I  A  A  R  E  K  F  Y                          SEQ ID No:20
      TCAACTTCAATGATTGCTGCTCGAGAAAAATTCTAT                          SEQ ID No:19
3'    AGTTGAAGTTACTAACGACGAGCTCTTTTTAAGATA      5'    7078.SL    SEQ ID No:18
```

Figure 19C. Oligonucleotides for construction of Hind III-BamH I *3' hmwC* fragment.

Hind III

L F T G D P R P L G K I L L K K T N E W K R K H L S K K * *

AGCTTTTACAGGCGACCCTCGTCCATTGGGCAAAATA 7079.SL SEQ ID No:21

CTGCTTAAGAAAACAAATGAATGGAAGCGGAAGCACTTGAGTAAAAAATAATAG 7080.SL SEQ ID No:22

AAAATGTCCGCTGGGAGCAGGTAACCCGTTTTATGACGAATTC 7082.SL SEQ ID No:25

TTTTGTTTACTTACCTTCGCCTTCGTGAACTCATTTTTTATTATCCTAG 7081.SL SEQ ID No:24

BamH I